

AMENDMENTS TO THE CLAIMS

1. (Currently amended) An infectious chimeric parainfluenza virus (PIV) comprising a major nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), a large polymerase protein (L), and a human PIV (HPIV) background genome or antigenome that is modified to encode a chimeric glycoprotein ~~incorporating one or more heterologous~~ comprising one or more structural domains, antigenic domains or epitopes of a glycoprotein of a first HPIV and one or more structural domains, antigenic domains or epitopes of a second heterologous, antigenically distinct HPIV.

2. (Previously Presented) The chimeric PIV of claim 1, wherein one or more heterologous genome segment(s) of the second, antigenically distinct HPIV encoding said one or more structural domains, antigenic domains or epitopes is/are substituted within the HPIV background genome or antigenome to encode said chimeric glycoprotein.

3. (Previously Presented) The chimeric PIV of claim 2, wherein said one or more heterologous genome segment(s) encode(s) one or more glycoprotein ectodomain(s) substituted for one or more corresponding glycoprotein ectodomain(s) in the background genome or antigenome.

4. (Previously Presented) The chimeric PIV of claim 2, wherein heterologous genome segments encoding both a glycoprotein ectodomain and transmembrane region are substituted for counterpart glycoprotein ecto- and transmembrane domains in the background genome or antigenome.

5. (Original) The chimeric PIV of claim 1, wherein said chimeric glycoprotein is selected from HPIV HN or F glycoproteins.

6. (Previously Presented) The chimeric PIV of claim 1, wherein the (HPIV) background genome or antigenome is modified to encode multiple chimeric glycoproteins.

7. (Previously Presented) The chimeric PIV of claim 1, wherein the HPIV background genome or antigenome is a partial HPIV3 genome or antigenome and the second, antigenically distinct HPIV is selected from HPIV 1 or HPIV2.

8. (Previously Presented) The chimeric PIV of claim 7, wherein the HPIV background genome or antigenome is a partial HPIV3 genome or antigenome and the second, antigenically distinct HPIV is HPIV2.

9. (Previously Presented) The chimeric PIV of claim 8, wherein one or more glycoprotein ectodomain(s) of HPIV2 is/are substituted for one or more corresponding glycoprotein ectodomain(s) in the HPIV3 background genome or antigenome.

10. (Previously Presented) The chimeric PIV of claim 9, wherein both glycoprotein ectodomain(s) of HPIV2 HN and F glycoproteins are substituted for corresponding HN and F glycoprotein ectodomains in the HPIV3 background genome or antigenome.

11. (Canceled)

12. (Original) The chimeric PIV of claim 10, which is further modified to incorporate one or more and up to a full panel of attenuating mutations identified in HPIV3 JS *cp45*.

13. (Canceled)

14. (Original) The chimeric PIV of claim 8, wherein PIV2 ectodomain and transmembrane regions of one or both HN and/or F glycoproteins is/are fused to one or more corresponding PIV3 cytoplasmic tail region(s).

15. (Original) The chimeric PIV of claim 14, wherein ectodomain and transmembrane regions of both PIV2 HN and F glycoproteins are fused to corresponding PIV3 HN and F cytoplasmic tail regions.

16. (Canceled)

17. (Previously presented) The chimeric PIV of claim 15, which is further modified to incorporate one or more and up to a full panel of attenuating mutations identified in HPIV3 JS *cp45*.

18. (Canceled)

19. (Original) The chimeric PIV of claim 1, which is further modified to incorporate one or more and up to a full panel of attenuating mutations identified in HPIV3 JS *cp45* selected from mutations specifying an amino acid substitution in the L protein at a position corresponding to Tyr942, Leu992, or Thr1558 of JS *cp45*; in the N protein at a position corresponding to residues Val96 or Ser389 of JS *cp45*, in the C protein at a position corresponding to Ile96 of JS *cp45*, a nucleotide substitution in a 3' leader sequence of the chimeric virus at a position corresponding to nucleotide 23, 24, 28, or 45 of JS *cp45*, and/or a mutation in an N gene start sequence at a position corresponding to nucleotide 62 of JS *cp45*.

20. (Previously Presented) The chimeric PIV of claim 1, wherein a plurality of heterologous genes or genome segments encoding antigenic determinants of multiple heterologous PIVs are added to or incorporated within the partial or complete HPIV background genome or antigenome.

21. (Previously Presented) The chimeric PIV of claim 20, wherein said plurality of heterologous genes or genome segments encode antigenic determinants from both HPIV 1 and HPIV2 and are added to or incorporated within a partial or complete HPIV3 background genome or antigenome.

22. (Previously Presented) The chimeric PIV of claim 20, wherein the chimeric genome or antigenome encodes a chimeric glycoprotein having structural domains, antigenic domains or epitopes from two or more different HPIVs.

23. (Original) The chimeric PIV of claim 1, wherein the chimeric PIV genome or antigenome is attenuated by addition or incorporation of one gene or cis-acting regulatory element from a bovine PIV3 (BPIV3).

24. (Original) The chimeric PIV of claim 1, wherein the chimeric PIV genome or antigenome incorporates one or more heterologous, non-coding non-sense polynucleotide sequence(s).

25. (Previously Presented) The chimeric PIV of claim 1, wherein the chimeric genome or antigenome encodes a chimeric glycoprotein having structural domains, antigenic domains or epitopes from both HPIV3 JS and HPIV1 or HPIV2.

26. (Original) The chimeric PIV of claim 1, wherein the chimeric genome or antigenome is modified by introduction of an attenuating mutation involving an amino acid substitution of phenylalanine at position 456 of the HPIV3 L protein.

27. (Original) The chimeric PIV of claim 26, wherein phenylalanine at position 456 of the HPIV3 L protein is substituted by leucine.

28. (Original) The chimeric PIV of claim 1, wherein the chimeric genome or antigenome incorporates one or more heterologous gene(s) or genome segment(s) encoding one or more respiratory syncytial virus (RSV) F and/or G glycoprotein(s) or immunogenic domain(s), fragment(s), or epitope(s) thereof.

29. (Previously presented) The chimeric PIV of claim, 1 which is a complete virus.

30. (Original) The chimeric PIV of claim 1 which is a subviral particle.

31. (Withdrawn, Currently amended) A method for stimulating the immune system of an individual to induce ~~protection against PIV~~ an immune response against one or more HPIVs which comprises administering to the individual an immunologically sufficient amount of the chimeric PIV of claim 1 combined with a physiologically acceptable carrier.

32. (Withdrawn) The method of claim 31, wherein the chimeric PIV is administered in a dose of 10^3 to 10^7 PFU.

33. (Withdrawn) The method of claim 31, wherein the chimeric PIV is administered to the upper respiratory tract.

34. (Withdrawn) The method of claim 31, wherein the chimeric PIV is administered by spray, droplet or aerosol.

35. (Withdrawn) The method of claim 31, wherein the background genome or antigenome is of human PIV3 (HPIV3) and the chimeric PIV elicits an immune response against HPIV 1 and/or HPIV2.

36. (Withdrawn) The method of claim 31, wherein the chimeric PIV elicits a polyspecific immune response against multiple human PIVs.

37. (Withdrawn) The method of claim 31, wherein a first, chimeric PIV and a second PIV are administered sequentially or simultaneously to elicit a polyspecific immune response.

38. (Withdrawn) The method of claim 37, wherein the second PIV is a second, chimeric PIV according to claim 1.

39. (Withdrawn) The method of claim 37, wherein the first, chimeric PIV and second PIV are administered simultaneously in a mixture.

40. (Withdrawn, Currently amended) The method of claim 37, wherein the first and second chimeric PIVs ~~are bear~~ comprise the same or different heterologous antigenic determinant(s).

41. (Withdrawn, currently amended) The method of claim 37, wherein the first chimeric PIV elicits ~~an~~ an immune response against HPIV3 and the second chimeric PIV elicits an immune response against HPIV 1 or HPIV2.

42. (Withdrawn) The method of claim 37, wherein the second chimeric PIV incorporates one or more heterologous gene(s) or genome segment(s) encoding one or more antigenic determinant(s) of respiratory syncytial virus (RSV).

43. (Withdrawn) The method of claim 42, wherein both the first and second chimeric PIVs elicit an immune response against RSV.

44. (Withdrawn) The method of claim 43, wherein the first chimeric PIV is administered initially in a vaccination protocol and the second chimeric PIV is administered subsequently in the vaccination protocol to provide initial immunization against HPIV3 and secondary immunization against HPIV1 or HPIV2 and to provide initial and secondary, booster immunization against RSV.

45. (Withdrawn) The method of claim 37, wherein the first, chimeric PIV incorporates at least one and up to a full complement of attenuating mutations present within PIV3 JS cp45 selected from mutations specifying an amino acid substitution in the L protein at a position corresponding to Tyr942, Leu992, or Thr1558 of JS cp45; in the N protein at a position corresponding to residues Val196 or Ser389 of JS cp45, in the C protein at a position corresponding to Ile96 of JS cp45, a nucleotide substitution in a 3' leader sequence of the chimeric virus at a position corresponding to nucleotide 23, 24, 28, or 45 of JS cp45, and/or a mutation in an N gene start sequence at a position corresponding to nucleotide 62 of JS cp45.

46. (Original) An immunogenic composition to elicit an immune response against PIV comprising an immunogenically sufficient amount of the chimeric PIV of claim 1 in a physiologically acceptable carrier.

47. (Original) The immunogenic composition of claim 46, formulated in a dose of 10^3 to 10^7 PFU.

48. (Original) The immunogenic composition of claim 46, formulated for administration to the upper respiratory tract by spray, droplet or aerosol.

49. (Original) The immunogenic composition of claim 46, wherein the chimeric PIV elicits an immune response against one or more virus(es) selected from HPIV1, HPIV2 and HPIV3.

50. (Original) The immunogenic composition of claim 46, wherein the chimeric PIV elicits an immune response against HPIV3 and another virus selected from HPIV1, HPIV2, and respiratory syncytial virus (RSV).

51. (Original) The immunogenic composition of claim 46, further comprising a second, chimeric PIV according to claim 1.

52. (Original) The immunogenic composition of claim 51, wherein the first chimeric PIV elicits an immune response against HPIV3 and the second chimeric PIV elicits an immune response against HPIV 1 or HPIV2, and wherein both the first and second chimeric PIVs elicit an immune response against RSV.

53. (Currently amended) An isolated polynucleotide comprising a chimeric PIV genome or antigenome which includes a human PIV (HPIV) background genome or antigenome modified to encode a chimeric glycoprotein ~~incorporating~~ comprising one or more heterologous structural domains, antigenic domains or epitopes of a first HPIV and one or more structural domains, antigenic domains or epitopes of a second heterologous, antigenically distinct HPIV.

54. (Previously Presented) The isolated polynucleotide of claim 53, wherein one or more heterologous genome segment(s) encoding the structural domains, antigenic domains, or epitopes of said second, antigenically distinct HPIV is/are substituted for one or more counterpart, genome segment(s) in the HPIV background genome or antigenome.

55. (Original) The isolated polynucleotide of claim 53, wherein, the chimeric genome or antigenome incorporates at least one and up to a full complement of attenuating mutations present within PIV3 JS *cp45*.

56. (Currently amended) A method for producing an infectious attenuated chimeric PIV particle from one or more isolated polynucleotide molecules encoding said PIV, comprising:

expressing in a cell or cell-free lysate an expression vector comprising an isolated polynucleotide comprising a background genome or antigenome modified to encode a chimeric glycoprotein ~~incorporating~~ comprising one or more ~~heterologous~~-structural domains, antigenic domains or epitopes of a first HPIV and one or more structural domains, antigenic domains or epitopes of a second heterologous, antigenically distinct HPIV, and PIV N, P, and L proteins.

57. (Original) The method of claim 56, wherein the chimeric PIV genome or antigenome and the N, P, and L proteins are expressed by two or more different expression vectors.

58. (Currently amended) An expression vector comprising an operably linked transcriptional promoter, a polynucleotide sequence which includes a background genome or antigenome modified to encode a chimeric glycoprotein ~~incorporating~~ comprising one or more ~~heterologous~~-structural domains, antigenic domains or epitopes of a first HPIV and one or more structural domains, antigenic domains or epitopes of a second heterologous, antigenically distinct HPIV, and a transcriptional terminator.

59. (Currently amended) An infectious chimeric parainfluenza virus (PIV) comprising a major nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), a large polymerase protein (L), and a background human PIV genome or antigenome that is modified to comprise at least one open reading frame that encodes a chimeric glycoprotein incorporating into a first glycoprotein open reading frame of a first PIV at least one gene segment encoding one or more counterpart ~~heterologous~~-structural domains, antigenic domains ~~and/or~~ or epitopes of a glycoprotein of a second heterologous, antigenically distinct PIV;

said at least one open reading frame that encodes a chimeric glycoprotein being inserted into the background PIV genome at one or more site(s) selected from the group consisting of a site between the P and M open reading frames, a site between the N and P open reading frames, a site between the HN and L open reading frames, and a site between the 3' leader sequence and the N open reading frame.

60. (Previously Presented) The infectious chimeric PIV of claim 59, in which one or more open reading frames encoding ectodomains of the heterologous glycoprotein are substituted for one or more counterpart open reading frames encoding ectodomains of the first glycoprotein.

61. (Previously Presented) The infectious chimeric PIV of claim 59 in which open reading frames encoding both an ectodomain and a transmembrane region of the heterologous glycoprotein are substituted for the counterpart open reading frames encoding an ectodomain and a transmembrane region of the first glycoprotein.

62. (Previously Presented) The infectious chimeric PIV of claim 59, in which open reading frames encoding an ectodomain and a transmembrane region of the heterologous glycoprotein are fused to open reading frames encoding a cytoplasmic tail region of the first glycoprotein.

63. (Previously Presented) The infectious chimeric PIV of claim 59, in which open reading frames encoding an ectodomain and a transmembrane region of HN or F or of both HN and F as the heterologous glycoprotein are fused to open reading frames encoding a cytoplasmic tail region of HN or F or of both HN and F, respectively, as the first glycoprotein.

64. (Previously Presented) The infectious chimeric PIV of claim 59, in which the recombinant genome or antigenome comprises a mutation producing an amino acid substitution of phenylalanine at position 456 of the HPIV L protein.

65. (Previously Presented) The chimeric PIV of claim 59, in which the recombinant genome or antigenome is further modified to incorporate one or more attenuating mutations

selected from the group consisting of mutations specifying an amino acid substitution in the L protein at a position corresponding to Tyr942, Leu992, or Thr1558 of JS *cp45*; in the N protein at a position corresponding to residues Val196 or Ser389 of JS *cp45*, in the C protein at a position corresponding to Ile96 of JS *cp45*, a nucleotide substitution in a 3' leader sequence of the chimeric virus at a position corresponding to nucleotide 23, 24, 28, or 45 of JS *cp45*, and/or a mutation in an N gene start sequence at a position corresponding to nucleotide 62 of JS *cp45*.

66. (Currently Amended) An isolated nucleic acid molecule comprising a background human PIV genome or antigenome that is modified to comprise at least one open reading frame that encodes a chimeric glycoprotein incorporating into a first glycoprotein open reading frame of a first PIV at least one gene segment encoding one or more counterpart ~~heterologous~~-structural domains, antigenic domains ~~and/or~~ or epitopes of a glycoprotein of a second heterologous, antigenically distinct PIV;

said at least one open reading frame that encodes a chimeric glycoprotein being inserted into the PIV background genome at one or more site(s) selected from the group consisting of a site between the P and M open reading frames, a site between the N and P open reading frames, a site between the HN and L open reading frames, and a site between the 3' leader sequence and the N open reading frame.

67. (Previously Presented) The isolated nucleic acid of claim 66, in which one or more open reading frames encoding ectodomains of the heterologous glycoprotein are substituted for one or more counterpart open reading frames encoding ectodomains of the first glycoprotein.

68. (Previously Presented) The isolated nucleic acid of claim 66, in which open reading frames encoding both an ectodomain and a transmembrane region of the heterologous glycoprotein are substituted for the counterpart open reading frames encoding an ectodomain and a transmembrane region of the first glycoprotein.

69. (Previously Presented) The isolated nucleic acid of claim 66, in which open reading frames encoding an ectodomain and a transmembrane region of the heterologous glycoprotein are fused to open reading frames encoding a cytoplasmic tail region of the first glycoprotein.

70. (Previously Presented) The isolated nucleic acid of claim 66, in which open reading frames encoding an ectodomain and a transmembrane region of HN or F or of both HN and F as the heterologous glycoprotein are fused to open reading frames encoding a cytoplasmic tail region of HN or F or of both HN and F, respectively, as the first glycoprotein.

71. (Previously Presented) The isolated nucleic acid of claim 66, in which the recombinant genome or antigenome comprises a mutation producing an amino acid substitution of phenylalanine at position 456 of the HPIV L protein.

72. (Previously Presented) The isolated nucleic acid of claim 66, in which the recombinant genome or antigenome is further modified to incorporate one or more attenuating mutations selected from the group consisting of mutations specifying an amino acid substitution in the L protein at a position corresponding to Tyr942, Leu992, or Thr1558 of JS *cp45*; in the N protein at a position corresponding to residues Val96 or Ser389 of JS *cp45*, in the C protein at a position corresponding to Ile96 of JS *cp45*, a nucleotide substitution in a 3' leader sequence of the chimeric virus at a position corresponding to nucleotide 23, 24, 28, or 45 of JS *cp45*, and/or a mutation in an N gene start sequence at a position corresponding to nucleotide 62 of JS *cp45*.

73. (Previously Presented) An expression vector comprising the isolated nucleic acid of claim 66 operatively linked to a promoter operative in a mammalian cell or *in vitro* and to a transcription terminator sequence operative in a mammalian cell or *in vitro*.

74. (Previously Presented) An immunogenic composition comprising an immunologically sufficient amount of the infectious chimeric PIV of claim 59 and a physiologically acceptable carrier.